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STUDIES ON THE BIOCHEMISTRY AND CHEMOTHERAPY OF TUBERCULOSIS.*

I. THE PERMEABILITY OF TUBERCLES FOR IODIN COMPOUNDS AND PROTEINS.

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GENERAL CONSIDERATIONS.

The principles of chemotherapy, as laid down by Ehrlich, are of so fundamental a character that there is no limit to their application in infectious diseases, and possibly in other conditions, notably cancer. With the spirilloses and trypanosome infections, in which most of the work has so far been done, the conditions are favorable for the meeting of the drug and the germ, since with most forms of these diseases the germ lives chiefly or entirely in the circulating fluids. It is noteworthy that the only disease in which "Therapia magna sterilisans" has been practiced successfully on an empirical basis is also a blood infection, malaria. The consideration of tuberculosis from the standpoint of chemotherapy brings in distinctly new problems owing to the fact that the bacteria are, in large part, located at points specifically removed from the circulation by proliferating tissues. The avascularity of the tubercle must of necessity have a large influence on the meeting of the drug and the germ, and this condition has perhaps been responsible for the lack of success of the innumerable empirical attempts at chemotherapy which have been made with the disease in the past. Avascularity of an infected tissue may, perhaps, make for either assistance or hindrance in chemotherapy, for we can imagine that the drug might accumulate in the avascular area, just as, for instance, calcium salts do, or, entering avascular and vascular tissues alike, it might remain longer where there is no circulation. Absence of living cells may also make a difference in that certain drugs may be either destroyed or activated by living cells, and hence have either

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a greater or a less effect in the necrotic portions of the tubercle than elsewhere in the body. These and other points present themselves, and to attack the problem of tuberculosis chemotherapy it would seem to be necessary to learn first to just what extent different classes of chemical substances enter tubercles, both early and advanced, how much they tend to accumulate specifically in the tissues, and how long they remain there. For a chemical which is to destroy the tubercle bacillus, it would seem, should be one that will enter readily into the avascular tuberculous lesions, and, if possible, enter or accumulate in such tissues more than in normal tissues.

The problem is further complicated by the chemical composition of the tubercle bacillus itself, with its large proportion of resistant fatty and waxy material, which must, it would seem, make its permeation and destruction a very different matter from the attack upon the naked and delicate trypanosomes, spirillae, and spirochaetes. Hence the permeability of the tubercle bacillus for chemicals of different classes becomes a fundamental question in connection with the main problem. In the investigation of the subject the fatty matter of the tubercle bacillus, while perhaps an obstacle to chemotherapy, makes attack of the problem appear somewhat easier, since the permeability of the bacteria must be largely determined by this substance which can be extracted from them in large amounts and rendered available for experimental work *in vitro*, without, at the beginning, calling for the extensive animal experimentation which is essential in the study of the chemotherapy of protozoan infections. The influence of the fatty constituents of the cells upon the permeability of tissue cells to drugs and dyes has already been extensively investigated, and we have, therefore, many clues for investigation of the permeability of *B. tuberculosis*.

In planning a systematic investigation on the chemotherapy of tuberculosis, therefore, it seemed desirable first to determine the entrance of various classes of substances into the tubercle and into the bacteria, since the effective tuberculocide must be, theoretically, one which enters freely and, if possible, selectively into the avascular tubercle, and with like facility passes through the fatty

sheath of the bacillus. We have found it possible to attack directly some of the problems involved, while others have called for preliminary studies of certain fundamental questions. Some of the work has advanced sufficiently to warrant a preliminary report, which should be introduced by stating that all the work reported in this and in subsequent articles has been done through the co-operation of several persons, each of whom has helped in various stages so that it is difficult to credit any particular step to any one or two persons. Those engaged in various aspects of the work here reported are Dr. Lydia DeWitt, Dr. H. J. Corper, Dr. G. L. Kite, Miss Hope Sherman, Mr. G. C. Lake, and ourselves.

ENTRANCE OF SUBSTANCES INTO TUBERCLES AND OTHER LESIONS.

HISTORICAL.

The only study we can find directly concerning itself with this topic is that of Oswald Loeb and Michaud.¹ A study of the distribution of iodine in normal animals had been made previously by Loeb,² who found that when injected in the form of KI, the brain, spinal cord, bone marrow, and fat tissue were usually free from iodine, the muscle contained very little, and then, in increasing amounts, the liver, lymph glands, kidneys, salivary glands, lungs, blood, and, of course highest of all, the thyroid. When compounds of iodine which are soluble in fat were injected (iodoform, ethyl iodide) it was found in the brain, spinal cord, and fat tissue. After ethyl iodide injection he found the iodine especially in the lungs, where it is excreted, while more iodine is found in the kidneys and salivary glands after KI injection, for the same reason.

When these same iodine compounds were injected into tuberculous rabbits and guinea-pigs, Loeb and Michaud found that regularly the tuberculous tissues took up a disproportionately large amount of iodine. Thus, four rabbits inoculated in one eye with tuberculosis showed from one and one-half to two and three-fourths times as much iodine in the tuberculous eyes as in the normal eyes, and tuberculous lungs were found to contain increasing amounts of iodine in proportion to the amount of tuberculous tissue they contained. Caseous lymph glands of guinea-pigs contained more iodine than any of the normal organs.

This important investigation has only recently begun to receive the attention it has deserved, and has as yet been neither confirmed nor extended so far as tuberculous lesions are concerned, although in view of the fact that these weighty conclusions rest upon a series of four rabbits and four guinea-pigs, and that the relatively inaccurate method of Baumann was used for the iodine determination, amplification is certainly required before entire acceptance is warranted.

Collateral support is given by two observations on cancer. Van den Velden³ reported the case of a man who died of gall duct cancer, with secondaries in the liver

¹ *Biochem. Ztschr.*, 1907, 3, p. 301.

² *Arch. exp. Path. u. Pharm.*, 1907, 56, p. 321.

³ *Biochem. Ztschr.*, 1908, 9, p. 54.

and pancreas, five and one-half hours after a subcutaneous injection of 3.0 gm. of NaI. Analysis showed iodine in abdominal, pleural, and pericardial fluids, and in two large secondary growths in the liver and pancreas, but none in the normal liver and pancreas tissue, despite the relatively avascular nature of the tumors. The absence of iodine in the normal tissues under these conditions is difficult to understand, and, in the light of our observations on animals, incredible. Takemura,¹ who found that iodine distributes itself in normal rats and mice much as Loeb found in guinea-pigs, (noting an especially high content in the skin), observed that in mice with cancer there is nearly as much iodine in the tumor tissue after injection of KI as in the tissues which normally contain the greatest amount of iodine; in sarcoma in rats the iodine in the tumor tissue was intermediate between iodine-rich and iodine-poor tissues. Our own experiments, as given below, indicate that these results may depend upon the amount of necrosis in the carcinomas and sarcomas. More recently Loeb² has reported the finding of a larger proportion of iodine in the enlarged glands removed by operation from a syphilitic (0.28–0.53 mg. per gm.) than in the blood of the same patient (0.082–0.088 mg.) 20 hours after the last dose of iodides.

In this connection might be mentioned the solitary observation of Loeb that the pus in a turpentine abscess in a rabbit injected with KI contained a larger proportion of iodine than the blood itself. Also, the now classical observation of Bondi and Jacoby³ that injection of rabbits causes more of the injected salicylic acid to localize in joints, even when there is no arthritis; and the earlier observation by Fillipi and Nesti⁴ that after aspirin has been given by mouth to persons with arthritis, the synovial fluid contains more salicylic acid than the urine. Other related observations are the following:

Blumenthal⁵ observed that the addition of iodine to the atoxyl molecule causes it to enter into inoculable sarcomas of dogs and rats with special avidity, although even in the uniodized state the atoxyl is found more abundantly in these tissues than in the normal tissues. The effect of the atoxyl is to cause an increased rate of growth in the tumors of these animals.

Kapsenberg⁶ states that an extract of tubercle bacilli made with water in the presence of chloroform, has a decided affinity for iodine, and that the resulting compound is specifically bactericidal for tubercle bacilli, but the data presented in this article are not sufficient to carry conviction.

Morel and Dalous⁷ injected tuberculous guinea-pigs with anthrax cultures and found that the anthrax bacilli do not enter the larger tubercles; but in small tubercles consisting only of a giant cell and a single row of epithelioid cells, the protoplasm of each of these may contain anthrax bacilli.

EXPERIMENTAL.

We have undertaken to repeat the experiments of Loeb and Michaud, and to amplify them. Our plan of procedure was as follows: Guinea-pigs, the largest obtainable, were injected subcutaneously with human tubercle bacilli (0.01 mg. usually), and the animals were selected, as far as possible, when they had the maximum enlargement of the regional lymph glands before ulceration led to evacuation of their contents. In

¹ *Ztschr. physiol. Chem.*, 1911, 72, p. 78.

² *Arch. exp. Path. u. Pharm.*, 1912, 69, p. 108.

³ *Hofmeister's Beitr.*, 1906, 7, p. 514.

⁴ *Allg. med. Zentralz.*, 1902, 71, p. 613.

⁵ *Deutsch. med. Wchnschr.*, 1910, 36, p. 2275.

⁶ *Berl. klin. Wchnschr.*, 1912, 49, p. 879.

⁷ *Compt. rend. Soc. de Biol.*, 1907, 62, p. 74.

order to secure local lesions which could be compared with corresponding normal tissues, several large males were inoculated in one testicle, but this did not give useful results, for invariably the other testicle developed extensive tuberculosis. Equally unsuccessful was the result of direct intra-hepatic inoculations, which caused only an extensive local miliary tuberculosis which rapidly became generalized. Good results were obtained by inoculating human tubercle bacilli into the vitreous of one eye, and then, just before the eyeball was ready to rupture, injecting the iodine compound and analyzing separately the normal and the tuberculous eye.

In order to learn whether the entrance of iodine compounds into tubercles depends upon some peculiarity of the tubercles themselves, or whether it is common to necrotic areas and exudates in general, a series of experiments was performed as follows: Necrosis of the entire left kidney was produced in rabbits by aseptic ligation of the artery, vein, and ureter. This is followed by a severe engorgement of the organ from the collateral circulation through the capsule, which results in stasis and total necrosis of everything but the capsule. Necrosis of muscle was produced by injecting 2 c.c. of 50 per cent formalin (equal to 20 per cent formaldehyde) into the muscle of one thigh; this also produces a severe local subcutaneous edema from which sufficient fluid could usually be expressed to permit of analysis. Exudates were produced by injecting into the left pleural cavity one to two gms. of aleuronat suspended in 5-10 c.c. water, sometimes with one c.c. of turpentine or a loopful of solid *Staphylococcus pyogenes aureus* culture added to produce more violent reactions. To secure an inert colloid mass to compare with the dead tissues, subcutaneous injections were made of sterile five per cent agar jelly, 8-20 c.c. being injected at 50° C. by means of a powerful syringe such as is used for cosmetic work with paraffin. Analysis of this agar showed it to contain a negligible amount of iodine, about 0.001 mg. per 20 c.c. in the amounts used. Several or all of these different procedures were carried out in the same animal in most instances, thus permitting a comparison of the iodine determinations in several different lesions as well as with the normal tissues of the same animal.

The injections were made subcutaneously with the following iodine compounds: Potassium iodide was used in five per cent solution, one c.c. of this solution (.050 gm. KI) generally being given per 100 gms. of animal weight as the standard dose. Iodoform was used in 10 per cent emulsion in olive oil, in doses of about one c.c. per 100 gms. animal weight. This was found to be more toxic than the other iodine compounds, especially for pregnant animals in which abortions usually resulted. Iodipin, 25 per cent iodine, was given in doses of about 0.5 c.c. per 100 gms. Ethyl iodide (Merck) was given in doses of about one c.c. per kilo. After the designated time had elapsed, the animals were bled from the carotids as thoroughly as possible, and in removing the tissues for analysis care was taken not to have errors arise from contamination with fluid from the site of the injection, which was always located as far as possible from the tissues that were to be examined. The tissues were finely ground in the fusion mixture, dried, and kept until ready for analysis. The large excess of alkali present in the fusion mixture seems, in view of the excellent results obtained in the subsequent analyses, to have been adequate to prevent any loss of volatile iodine compounds, even of the highly volatile ethyl iodide.

At first we made our analyses by the method devised by Hunter,¹ which possesses very evident advantages over the Baumann method, in that exact titration is used instead of the colorimetric method with its large subjective element, and especially

¹ *Jour. Biol. Chem.*, 1910, 7, p. 321.

in that it involves estimation of six times the amount of iodine originally present, which enormously reduces the error in determining iodine in small fractions of a milligram, as is necessary in this work. We soon found, however, that while we had no trouble in obtaining accurate results in our trial analysis of thyroid tissue or of test mixtures, yet during actual series of analyses there frequently occurred serious errors, sometimes total loss and sometimes large excess. In a number of instances these errors destroyed a large amount of work because they involved essential members of a series where duplicates were impossible. Investigation showed that there are several possible sources of error in this method. These have also been noted by others, especially by F. C. Kendall of New York, who published a preliminary report of an improved method¹ and who very kindly furnished us with a detailed account of his observations before their publication. Not securing altogether satisfactory results even with this method, a systematic investigation of the sources of error and the best means of correcting them was made by one of us (Hedenburg) and a method was at last devised which has been found altogether reliable and which possesses the aforementioned advantages of Hunter's method. This method will soon be described in detail in another publication.

The main results of our experiments are given in Tables 1 and 2, in which the figures represent milligrams of iodine per gram of fresh weight of tissue or fluid. Where more than one figure is given, without explanation, the results of duplicate or triplicate analysis are concerned, and these indicate well the limits of accuracy of the methods used. A dash indicates that no analysis was made. A question mark indicates that there is doubt as to the accuracy of the result obtained.

DISCUSSION OF RESULTS.

Examination of Tables 1 and 2 discloses the following facts: The methods of analysis used are sufficiently delicate and accurate nearly always to give reliable results, even with the small fractions of milligrams of iodine present in many of the samples analyzed. Occasionally, a result was obtained which was obviously entirely incorrect, and this is indicated in the table by a question mark. Such errors were observed chiefly when the original Hunter method was used, rarely with the newly devised modification of Hedenburg.

The relative amount of iodine found in the various tissues and organs seemed to show some variation with the form in which the iodine was compounded when injected, whether water-soluble KI or lipid-soluble CHI_3 , iodipin or ethyl iodide. But the results are not altogether in harmony with the observations of Loeb and others on the organotropic character of the partition of different forms of iodine compounds. We do not attempt to interpret these disagreements, as our problem lies elsewhere, but give the figures without

¹ *Proc. Soc. Exp. Biol. and Med.*, 1910, 8, p. 120; complete report in *Jour. Amer. Chem. Soc.*, 1912, 34, p. 894.

further comment. In a few experiments, extraction was performed with absolute ether, and the tissues were prepared for extraction by grinding to powder with anhydrous sodium sulfate. The ether extract was then shaken out with water to remove any traces of iodides and evaporated to dryness after mixing with fusion mixture. It will be noted that a little iodine was found in the brain and fat tissues even when it was given in the form of KI. No differences were observed between the results with rabbits and with guinea-pigs.

Potassium iodide given subcutaneously is eliminated rapidly, so that there is very little left in the blood or tissues even 12 hours later. With iodoform and iodipin very irregular results were obtained, which perhaps depend upon local conditions modifying absorption. Thus, in an experiment with iodipin, none at all could be found in the blood or organs of one animal (19, Table 1), while other animals with somewhat larger doses showed considerable amounts. Also, of three guinea-pigs given iodoform in much the same way, the amount of iodine in the blood was respectively 0.205, 0.006, and 0.120 mg. per cubic centimeter of blood. We have not attempted to determine how large a proportion of the iodine found in the blood and tissues is in the form injected and how much is inorganic iodides.

The blood practically always contains more iodine, no matter in what compound it is given, than any tissue or organ, whether normal or otherwise. Occasionally during active excretion the kidney will be found to contain more iodine than the blood, and generally it contains nearly as large a proportion weight for weight, as the blood of the same animal. The relation is well shown in the following parallel columns from a series of analyses under various conditions of dosage, time, iodine compound, and kind of animal used (guinea-pigs and rabbits).

On account of its function of excreting iodine the kidney cannot be compared with other tissues. Of the chief viscera, the liver seems to be perhaps the most stable as to the proportion of iodine, but as a rule it contains much less iodine than the blood, generally about one-third as much per gram. Thus of 29 analyses under the varied conditions of these experiments, in three the liver contained approximately the same amount of iodine as the blood, in four it contained

TABLE I.
IODIN IN NORMAL AND TUBERCULOUS TISSUES.

Animal	Dosage	Interval between Injection and Death	Blood	Liver	Spleen	Kidneys	Lungs	Tuberculous Glands	Miscellaneous	Autopsy Findings and Remarks
1. (Guinea-pig) 770 gms.	0.385gm. KI	6 hrs.	?	0.064 (left) 0.072 (right)	0.094	0.115	?	0.092	Testes, 0.110 Urine, 19.0 mg.	KI injected intravenously (in all other experiments subcutaneous). Lymph glands hard, large, full of small tubercles, some caseous; analyzed together, wt. 5.5 gms. Spleen filled with tubercles, many smaller ones in liver, a few in lungs.
2. " 600 gms.	0.300gm. KI	48, 24, and 6 hrs.	0.170 0.218	?	0.074	0.167	0.151	0.160	Testes, 0.121	Glands large and caseous in inguinal region, hard and large in mediastinal, perivascular, and pancreatic; analyzed together, wt. 6 gms. Many 2-3 mm. tubercles in spleen, smaller ones in liver.
3. " 480 gms.	0.250gm. KI	48, 24, and 6 hrs.	0.337	0.108 (left) 0.110 (right)	0.071	0.034	0.147	0.203	Inguinal glands only slightly enlarged and caseous, pancreatic and mediastinal enlarged and hard; analyzed together, wt. 7.6 gms. Extensive miliary tubercles in liver and lungs, larger in spleen. Bled incompletely.
4. " 400 gms.	0.200gm. KI	72, 48, 24, and 6 hrs.	0.388 0.433	0.165 (left) 0.433 (right)	lost	0.518	0.348	0.295 0.481 pus	Inguinal glands contained softened material, analyzed separately (0.9 gm.) from the rest of the enlarged glands (5.5 gms.). Many 1-2 mm. tubercles in spleen, a few in lungs and liver.
5. " 720 gms.	0.350gm. KI	48, 24, and 6 hrs.	0.680 0.430	0.567 0.552 0.635	0.108	1.168	0.213	0.285 wall 0.790 pus	Large caseous axillary glands containing 3.3 gms. softened material analyzed separately from the wall of the cavity, wt. 2.2 gms.
6. " 370 gms.	0.2 gm. KI	6 and 3 hrs.	0.558 0.479	0.161 0.132 (left)	0.309	Large caseous cervical glands.
7. (Rabbit) 1,750 gms.	0.9 gm. KI	4 hrs.	0.472	0.133 (right)	0.290	Eye, normal 0.220 tuberculous	Extensive tuberculosis of choroid, this eye weighing 4.5 gms. and containing 1.7 mg. iodine; normal eye wt. 2.2 gms.; iodine 0.48 mg. No tuberculosis elsewhere.
8. " 1,500 gms.	0.750 gm. KI	6 hrs.	0.171 0.168	0.025 (left) 0.035 (right)	0	0.151	0.031	Brain 0.020 Fat 0.032	Moderate tuberculosis of choroid, eye weighing 3.6 gms. and containing 0.541 mg. iodine; normal eye wt. 2.7 gms.; iodine 0.492 mg. No tuberculosis elsewhere.
9. " 1,800 gms.	0.9 gm. KI	8 hrs.	0.104 0.179	0.042 (left) 0.040 (right)	0.003	Eye, normal 0.078 tuberculous	Tuberculous eye weighed 4.0 gms. iodine 0.67 mg.; normal eye wt. 3.2 gms.; iodine 0.25 mg. No tubercles in other organs.
10. " 1,500 gms.	0.750 gm. KI	12 hrs.	0.006 0.011	0	0	0	0	Brain 0 Fat 0.015 No iodine in either eye	Normal eye wt. 2.8 gms., tuberculous, wt. 4.8 gms. Not sufficient iodine to be detected in any of the organs.

11. (Guinea-pig) 550 gms. . .	5.5 c.c. 10 per cent CHI ₃	48 hrs.	0.128 (left) 0.071 (right) ?	0.100	0.111	0.100	0.152	Pregnant, died without being bled. Large caseous inguinal glands, 3.0 gms. Many miliary tubercles in spleen, a few in liver and lungs.
12. " 680 gms. . .	6.8 c.c. 10 per cent CHI ₃	72 and 24 hrs.	0.093 0.093	0.060 0.033 tubercles	0.079	0.118	0.087	0.126	Muscle, 0.064	Bled fairly well. Large partly caseous and inguinal and hard pancreatic and mediastinal glands analyzed together, wt. 6.1 gms. Liver very fatty. Spleen full of tubercles.
13. " 800 gms. . .	4 c.c. 10 per cent CHI ₃	6, 5, 3 days	0.205	0.002 0.002	0.013	?	0.090	0.013	Fat	Advanced pregnancy. Not well bled. Some large caseous areas isolated from rest of liver tissue and analyzed separately (0.8 gm.). Liver fatty. Spleen full of tubercles.
14. " 600 gms. . .	3 c.c. 10 per cent CHI ₃	5 and 3 days	0.006	0.025 0.002 ether extract	0	0	0.003	0.007 capsule 0.013 pus ether extract	Testes Fat	Inoculated in left testicle; both testicles found full of small tubercles. Few tubercles in liver. Large abscess in left axilla (pseudotuberculosis?) the pus of which was extracted with ether and extraction and residue analyzed separately.
15. (Rabbit) 1,600 gms. . .	5 c.c. 10 per cent CHI ₃	5 and 3 days	0.120 0.121	0.172 (left) 0.180 (right)	Eye, normal tuberculous	Inoculated in right eye, which was nearly ready to rupture, wt. 4.8 gms., iodine 1.28 mg.; normal eye wt. 4.0 gms., iodine 1.15 mg. No tuberculosis elsewhere.
16. " 2,000 gms. . .	10 c.c. 10 per cent CHI ₃	40 hrs.	0.217	0.080 (left) 0.053 (right)	0.126	Eye, normal tuberculous	Died from iodoform, and not bled, therefore all organs full of blood. Normal eye, wt. 3.0 gms., total iodine 0.279 mg.; tuberculous eye wt. 4.8 gms.; iodine 0.578 mg. No tuberculosis elsewhere.
17. (Guinea-pig) 550 gms. . .	3 c.c. iodipin	72, 36, and 12 hrs.	0.019 0.031	0.093 (left) 0.091 (right)	0.061	0.195	0.032	0.111	Brain Fat	Inguinal glands very large and caseous, some caseation in pancreatic and mediastinal glands; analyzed together, wt. 11.5 gms. Spleen almost entirely tuberculous, slight in liver and lungs. Thoroughly bled. Glands showed only slight caseation; wt. together 4.9 gms. Not much tuberculosis in other organs. Bled thoroughly.
18. " 600 gms. . .	3 c.c. iodipin	72 and 24 hrs.	0.082	0.009 (left) 0.008 (right)	0.052	0.016	0.086	0.044	Inoculated in testes. Extensive tuberculosis in all organs, especially lungs. No iodine could be found anywhere.
19. " 600 gms. . .	1 c.c. iodipin	4, 3 and 1 day	0	0.009 (left) 0.008 (right)	0	0	0	0	Testes	Tuberculous eye wt. 5.2 gms., iodine 0.033 mg.; normal eye 2.0 gms., iodine 0.015 mg. No other tuberculosis.
20. (Rabbit) 1,750 gms. . .	4.5 and 2.25 c.c. iodipin	3 and 1 day	0	0.042 (left) 0.036 (right)	0.021	Eye, normal tuberculous	Normal eye wt. 2.2 gms., iodine 0.062 mg.; tuberculous eye 5.8 gms., iodine 0.86 mg. No tuberculosis elsewhere.
21. " 1,700 gms. . .	2.5 c.c. ethyl iodid	6 hrs.	0.215 0.207	0.009 (left) 0.008 (right)	0.080	Brain Fat	Normal eye wt. 2.2 gms., iodine 0.062 mg.; tuberculous eye 5.8 gms., iodine 0.86 mg. No tuberculosis elsewhere.

TABLE 2.
IODIN IN NECROTIC TISSUES AND EXUDATES.

Animal	Dosage	Interval between In- jection and Death	Blood	Liver	Spleen	Kidneys	Lungs	Agar	Muscle	Exudate	Miscellaneous	Autopsy Findings and Re- marks
1. (Guinea-pig) 370 gms..	0.38 gm. KI	6 hrs.	0.488	0.101	0.139	0.248	0.312	0.265	0.087	0.221	Bile 0.314	8 c.c. agar injected subcutane- ously 60 hrs. before bleed- ing, which was incomplete. Agar found well encapsu- lated; much edema in sur- rounding tissue, which was analyzed separately.
2. " 450 gms..	0.45 gm. KI	6 hrs.	?	0.077 (left) 0.099 (right)	0.107	0.111	0.112	0.100	Bile 0.310 Glands 0.218	Duplicate of No. 1, but not bled until 6 days after agar injection.
3. " 720 gms..	0.35 gm. KI	48, 24, and 6 hrs.	0.680	0.635 (left) 0.552 (right)	0.108	1.168	0.213	0.280	Capsule about agar 0.300	Duplicate of Nos. 1 and 2, bled after 9 days.
4. (Rabbit) 3,450 gms...	1.5 gm. KI	18 and 1 hr.	0.812	0.485 (left) 0.513 (right)	0.365	0.679 (left) 0.420 (right)	0.429	0.435	0.139	Abscess pus 0.503	Left kidney ligated 3 days and agar injected 9 days before death, and also agar and staphylococci to pro- duce subcutaneous abscess. Died without being bled. Advanced pregnancy. Left kidney entirely necrotic, wt. 23.2 gms., contained 15.7 mg. iodine; right kid- ney wt. 11.4 gms.; iodine 4.8 mg. Sterile agar well encapsulated; infected agar in abscess cavity.
5. " 2,140 gms...	1.06 gm. KI	48, 24, and 6 hrs.	0.524 0.536	0.187 (left) 0.188 (right)	0.317	0.487 (left) 0.500 (right)	0.095	Hematoma in muscle 0.352	Left kidney ligated 5 days before death. Bled only fairly thoroughly. Left kidney entirely necrotic, wt. 11.2 gms.; iodine 5.4 mg.; right kidney 5.2 gms.; iodine 2.6 mg. Hema- toma in psoas muscle analyzed separately.

6. (Rabbit) 2,470 gms...	1.24 gm. KI	25 and 6 hrs.	0.425	0.114 (left) 0.131 (right)	0.178	0.539 (left) 0.389 (right)	0.040? (left) 0.147 (right)	0.061	0.159	Left kidney ligated 5 days before bleeding; 3 days later injected auronat into left pleural cavity. Bled well. Much turbid exudate and fibrin in pleura, analyzed together, wt. 12 gms. Left lung collapsed and congested. Left kidney necrotic, wt. 14.2 gms.; iodine 6.7 mg.; right wt. 9 gms.; iodine 3.5 mg. Duplicate of Nobbi No. 6 poorly. Resected No. 6 at autopsy. Left kidney 9.7 gms., iodine 2.6 mg.; right, 6.6 gms., iodine 2.1 mg.
7. " 1,500 gms...	0.75 gm. KI	24 and 6 hrs.	0.391	0.681 (left) 0.894 (right)	0.221	0.067 (left) 0.319 (right)	0.184	0.343	Kidney ligated 12 days; formalin injected into muscle 4 days and auronat - staphylococcus suspension into left pleura 2 days before bleeding thoroughly. Heavy fibrinopurulent pleuritis and pericarditis; separated fibrin from fluid and analyzed separately. Left lung collapsed. Left kidney necrotic, wt. 30.5 gms., iodine 12.3 mg.; right kidney 8.2 gms., iodine 4.35 mg. Muscle necrotic where formalin injected; much subcutaneous edema fluid, analyzed separately. Focal necrosis in liver.
8. " 2,000 gms...	1.0 gm. KI	22 and 6 hrs.	0.303 0.306	0.095 (left) 0.105 (right)	0.123	0.403 (left) 0.530 (right)	0.326 (left) 0.186 (right)	0.030 normal 0.300 necrotic	0.310 edema 0.361 pleura 0.461 fibrin in pleura	Agar injected 9 days before bleeding. Necrosis of skin followed first KI injection. Duplicate of No. 9, but killed 1 day later.
9. (Guinea-pig) 620 gms	0.31 gm. KI	3 days, 29 hrs.	0.0	0.007	0.010	?	0.011	0.020	Fat 0.030 Brain 0.030	
10. " 600 gms	0.3 gm. KI	4 and 2 days	0.005 0.006	0.004	0.079?	?	0.016	0.022	

TABLE 2—Continued.

Animal	Dosage	Interval between injection and Death	Blood	Liver	Spleen	Kidneys	Lungs	Agar	Muscle	Exudate	Miscellaneous	Autopsy Findings and Remarks
11. (Rabbit)	1,400 gms... 0.35 gm. KI	36 and 12 hrs.	0.016 0.023	0.008 0.007	?	0.020 (left) 0.017 (right)	0.016	0.916?	0.004 normal 0.017 necrotic	0.020 pleura 0.014 edema	Kidney ligated 8 days, aleuronat injected subcutaneously and formalin injected into muscle 3 days, agar injected 6 days before bleeding thoroughly. Aleuronat found encapsulated. Muscle necrotic and much subcutaneous edema where formalin was injected; fluid analyzed separately. Left kidney wt. 11.9 gms., lodin 0.24 mg.; right 6.9 gms., lodin 0.07 mg.
12. "	1,800 gms... 5 c.c. 10 per cent CHI ₃	3 days	0.014	0.021 (left) right lost	0.012	0.001 normal 0.001 necrotic	Brain, ether extract o Residue 0.025	Kidney ligated 6 days. Agar injected 5 days; emulsion of aleuronat and turpentine injected into pleura, and formalin into leg 3 days before death. Not bled. Brain extracted with ether. Duplicate of 12, except lived 2 days longer and then was bled to death. Severe left pleurisy, with atelectasis of left lung. Liver very fatty. Muscle necrosed by formalin. Left kidney wt. 12.8 gms., lodin 1.26 mg.; right 6.9 gms., lodin 0.47 mg.
13. "	1,700 gms... 5 c.c. 10 per cent CHI ₃	6 days	0.072 0.072	0.032	?	0.009 (left) 0.006 (right)	0.088 (left) 0.071 (right)	0.077	0.013 normal 0.100 necrotic	0.078 pleura	Bile Fat 0.076	Ligated kidney, 8 days, agar injected 7 days, aleuronat and turpentine emulsion injected into chest-wall and formalin injected into leg 3 days before being bled to death. Much subcutaneous edema near formalin-killed muscle. Left kidney necrotic, wt. 11.4 gms., lodin 0.009 mg.; right 6.2 gms., lodin 0.012 mg.
14. "	1,500 gms... 3 c.c. 25 per cent iodipin	6 days	0.006 0.007	0.002 0.002	o	0.001 (left) 0.002 (right)	0.001	0.010	0.0 normal 0.001 necrotic	0.0035 pleura 0.0025 edema	Brain Fat o 0.005	

15. (Rabbit)	1,750 gms...	1.75 c.c. ethyl iodid	6 hrs.	0.168 0.161	0.060 0.052	0.030	0.066 (left) 0.250 (right)	0.043	0.072	0.018 normal 0.052 necrotic	0.105 aleu- ronat 0.130 edema	Brain 0.001 residue Ether extract 0	Duplicate of No. 12. Aleu- ronat injected into Peri- toneum and removed as solid dry lump. Brain re- moved and ether extract analyzed. Left kidney wt. 1.1 gms., iodine 0.8 mg.; right 5.1 gms., iodine 1.3 mg.
16.	" 2,000 gms...	2 c.c. ethyl iodid	6 hrs.	0.136 0.124	0.039 0.042 0.020 ether extract	0.026 (left) 0.120 (right)	0.019 (left) 0.020 (right)	0.024	0.012 normal	0.090 fluid 0.006 solid	Brain, ether extract 0	Duplicate of No. 15, except aleuronat and turpentine emulsion was injected into left pleura, causing much exudate and collapse of left lung and some solidifica- tion, fibrin of exudate analyzed separately from fluid. Liver showed exten- sive focal necrosis. Left kidney, wt. 14.8 gms., iodine 0.4 mg.; right 6.2 gms., iodine 0.75 mg.
17.	" 2,500 gms...	2.5 c.c. ethyl iodid	12 hrs.	0.433	0.102 (left) 0.097 (right)	0.079	0.214 (right) left lost	0.320 (left) 0.230 (right)	0.283	0.040 normal 0.004 necrotic	0.336	Brain 0.021	Duplicate of No. 16 but died when bleeding was started, and therefore incompletely bled. No focal necrosis of liver.

a very little more and this when there was but very little iodine present (three were iodipin and one KI experiments); while of the 22 in which the blood contained the most iodine, in 16 the excess was in a ratio between two and four to one.

TABLE 3.
RELATIVE IODINE CONTENT OF KIDNEYS AND BLOOD.

	Blood	Kidney	Injection	Time Elapsed after Injection
1.....	.488	.248	KI	6 hours
2.....	.680	1.168	KI	48, 24, and 6 hours
3.....	.812	.420	KI	18 and 1 hour
4.....	.524	.500	KI	48, 24, and 6 hours
5.....	.425	.389	KI	24 and 6 hours
6.....	.391	.319	KI	24 and 6 hours
7.....	.306	.530	KI	22 and 6 hours
8.....	.005	.000	KI	4 and 2 days
9.....	.019	.011	KI	36 and 12 hours
10.....	.093	.118	CHI ₃	72 and 24 hours
11.....	.072	.069	CHI ₃	6 days
12.....	.025	.195	iodipin	72, 36, and 12 hours
13.....	.060	.016	iodipin	72 and 24 hours
14.....	.130	.120	C ₂ H ₅ I	6 hours
15.....	.432	.214	C ₂ H ₅ I	12 hours
16.....	.168	.256	C ₂ H ₅ I	6 hours

The statement of Loeb that the left lobe of the liver regularly contains less iodine than the right we can corroborate in part only. In only two of 19 livers examined was there appreciably more iodine in the left, and in 10 there was definitely more in the right. In the remainder there was no difference above the limit of error of analysis. Generally, the ratio varies between 5:6 and 7:8. The two exceptional results were obtained with iodoform and iodipin.

As a rule, we found less iodine in the lungs than in the liver, but often the amount is about the same, and it is not uncommon to find more in the lungs, especially when only traces are left in the body. In four experiments when ethyl iodide had been given, we observed an excess of iodine in the lungs in two, and in the liver in two, which does not entirely corroborate Loeb, but our figures are too few to be significant.

The figures for the spleen vary greatly, perhaps because of the small quantity of material available for analysis; all in all it ranked about the same as in the liver. Herein we fail to corroborate Boruttau, who states that lymphatic tissues take on an excess of iodine, but corroborate Loeb (1912).

In all cases the muscle content runs far below that of all the

other tissues, except the brain, containing usually but one-half to one-third as much as the liver. Thus, the average of 12 analyses of liver and muscle from the same animals under varying conditions, showed 0.115 mg. iodine per gram liver and 0.041 mg. per gram muscle. In only two cases did the muscle have as much as half the amount present in the liver. The testicles seem to take on about as much iodine as the liver, and apparently the bile is an important avenue of escape of iodine from the blood.

The effects of pathological changes upon the tissues were very definite. Tuberculous lymph glands do, as Loeb first showed, take up in general relatively more iodine from the blood than do the liver, spleen, and lungs of the same animal. Thus, in nine of 11 experiments the tuberculous lymph glands contained more iodine than the liver, and in the best experiments with KI the amount approaches that in the blood. See Table 4.

TABLE 4.
IODINE IN CASEOUS GLANDS.

No. in Table 1	Blood	Liver	Caseous Glands	Injection	Time after Injection
1.....	?	.068	.092	KI	6 hours
2.....	.195	?	.160	KI	48, 24, and 6 hours
3.....	.337	.109	.203	KI	48, 24, and 6 hours
4.....	.408	.400	.481	KI	78, 42, 24, 6 hours
5.....	.550	.580	.790	KI	48, 24, and 6 hours
11.....100	.152	CHI ₃	48 hours
12.....	.093126	CHI ₃	72 and 24 hours
13.....	.205	.060	.013	CHI ₃	6, 5, and 3 days
14.....	.006	.002	.013	CHI ₃	5 and 3 days
17.....	.025	.065	.111	iodipin	72, 36, and 12 hours
18.....	.082	.092	.044	iodipin	72 and 24 hours
19.....	.0	.0	.0	iodipin	4, 3, and 1 day

It is especially noticeable that when the caseous material was abundant enough to permit of separation from the rest of the gland substance, it contained much more iodine than did the non-caseous portion of the glands, as seen in experiments Nos. 4, 5, and 14, where the figures are:

	4	5	14
Gland substance.....	0.295	0.285	0.007
Caseous contents.....	0.481	0.790	0.013

In only a few instances was there a noticeable deficit in iodine in the tuberculous tissues. In experiment No. 13 a small amount of necrotic liver tissue (0.9 gm.) seemed to contain less iodine than the

rest of the liver, but the amount of iodine involved is so small that the results are of doubtful reliability. The fact that here and in the glands in Nos. 13 and 18 the amount of iodine is lower in the caseous tissue than in the normal liver, may be ascribable to a relative chemotropism of the liver for iodoform and iodipin used in these experiments.

Tuberculous lesions in the eye show, as was also found by Loeb and Michaud in four experiments, an increased capacity for taking up iodine, as shown by the following summary from Table 1.

TABLE 5.
IODINE IN TUBERCULOUS AND NORMAL EYES.

No. in TABLE 1	WEIGHT OF EYE		TOTAL IODINE		MG. IODINE PER GRAM		FORM OF IODINE INJECTED
	Normal	Tuberculous	Normal	Tuberculous	Normal	Tuberculous	
7.....	2.2	4.5	0.48	1.7	0.220	0.381	KI, 4 hours
8.....	2.7	3.6	0.49	0.54	0.182	0.150	KI, 6 hours
9.....	3.2	4.0	0.25	0.67	0.078	0.166	KI, 8 hours
10.....	2.8	4.8	0	0	0	0	KI, 12 hours
15.....	4.0	4.8	0.15	1.38	0.038	0.267	Iodoform
16.....	3.0	4.8	0.28	0.53	0.093	0.117	Iodoform
20.....	2.0	5.2	0.015	0.033	0.0075	0.006	Iodipin
21.....	2.2	5.8	0.062	0.86	0.028	0.148	Ethyl iodid
Average.....	2.76	4.7	0.216	0.720	0.081	0.154	

Of these eight experiments, without exception the amount of iodine is greater in the tuberculous eye than in the normal eye, although in two (8 and 20) the proportion of iodine is slightly greater in the normal eye. Taken all together, there is over three times as much iodine in the tuberculous eyes, and nearly twice as large a proportion. The low figure for iodipin (No. 20) corresponds entirely with the proportion of iodipin in the liver of the same animal (0.008), and it is evident that after injections of iodoform and ethyl iodid the iodine readily enters the eyes, especially the tuberculous eyes, although whether as organic or inorganic compounds we have not ascertained.

That the entrance of iodine into tuberculous tissue is not characteristic of tuberculosis is established by the analyses of the tissues of animals in which necrosis and exudates were produced experimentally (Table 2). In 10 rabbits which had the left kidney rendered totally necrotic by ligation of all the blood vessels, there

is found to result a great increase in the size of the organ, from an average of 7.2 gms. to 15.3 gms., because of hemorrhage and edema. In spite of the avascularity of these kidneys, iodine permeates them rapidly, so that six hours after injection there is found to be, on the average, almost identically the same proportion of iodine in the avascular necrotic kidney and in the normal kidney, a proportion which, as pointed out previously, approximates that of the iodine content of the blood more closely than in any other organ. These facts are shown in Table 6, summarized from Table 2. Therefore, it seems evident that in a short time, the iodine in the blood will penetrate even so large an avascular area as an entire kidney, and reach practically the same concentration as in the blood itself.

TABLE 6.
IODINE IN NORMAL AND NECROTIC KIDNEYS.

No. in Table 2	Gms. Weight of Kidneys		Total mg. Iodine in Kidneys		mg. Iodine per gm. of		
	Necrotic	Normal	Necrotic	Normal	Blood	Necrotic Kidney	Normal Kidney
4.....	23.2	11.4	15.7	4.8	.812	.679	.420
5.....	11.2	5.2	5.4	2.6	.530	.487	.500
6.....	14.2	9.0	6.7	3.5	.425	.539	.389
7.....	9.7	6.6	2.6	2.1	.391	.267	.310
8.....	30.5	8.2	12.3	4.4	.305	.403	.530
11.....	11.9	6.9	0.2	0.1	.010	.020	.011
13.....	12.8	6.9	12.6	4.7	.072	.099	.069
15.....	12.1	5.1	10.8	1.3	.168	.066	.256
16.....	14.8	6.2	0.4	0.8	.130	.026	.120
Total.....	140.4	65.5	66.7	24.3	2.852	2.586	2.614
Average.....	15.6	7.3	7.4	2.7	.317	.287	.290

Of all the tissues, however, the normal kidney alone seems to be so permeable for iodine that it comes to contain the same proportion as the blood, a fact which is presumably related to the functional activity of the organ. If we take another tissue which is not normally so permeable for iodine, such as the muscle, we find the interesting fact that necrotic areas in this tissue also tend to contain approximately as much iodine as the blood of the same animal, while the normal muscle tissue, in spite of its much greater blood supply, contains much less iodine. This fact is shown in Table 7.

In the above series the necrosis was produced by injection of strong formalin (except No. 5, in which trauma during operation

probably caused the injury). In removing the tissues at autopsy, the necrosis not being sharply circumscribed, more or less normal tissue was probably always included with the necrotic muscle; if only completely necrotic muscle had been present in these samples, it is probable that the proportion of iodine would have been still higher.

TABLE 7.
IODINE IN NORMAL AND NECROTIC MUSCLE.

No. in Table 2	Blood	Normal Muscle	Necrotic Muscle
1.....	.488	.087
4.....	.812	.139
5.....	.530	.095	.352
6.....	.425	.061
8.....	.305	.030	.300
11.....	.019	.004	.017
12.....001	.091
13.....	.072	.013	.100
14.....	.006	.000	.001
15.....	.165	.018	.052
16.....	.136	.012
17.....	.433	.040	.064
Average of analyses where all three figures were obtained.....	.219	.029	.126

The explanation of these results, it seems to us, must be as follows: The partial impermeability of living cells, which presumably differs in all organs and cells, is destroyed when the cell is killed. Therefore, the readily diffusible iodine compounds present in the blood and tissue fluids will diffuse into the necrosed tissue elements just as they would into any inert water-filled colloidal mass, with the resulting tendency, as shown by our figures, to approach osmotic equilibrium of iodine in blood and necrotic tissue. The large amount of iodine present in necrotic tissues, whether tuberculous or otherwise, is, therefore, dependent on purely physical conditions, i.e., the destruction of the semi-permeability of the cells. That it does not depend upon any chemical attraction, or even a specific physical "adsorption," is shown by the fact that if some time is allowed for the iodine to be excreted in part from the body after injection, it leaves the necrotic tissues, the blood and the normal tissues *pari passu*. Support is given to this interpretation by the results of implantation of agar into the subcutaneous tissue, followed by injection of iodine compounds at various intervals. The agar was introduced at a temperature of about 50° C.,

and solidified in a lump which soon became encapsulated, and after a time permeated by invading strands of granulation tissue. The results of analyses from several such experiments are given in Table 8.

TABLE 8.
IODIN IN AGAR.

No. in Table 2	Blood	Liver	Agar
1.....	.488	.101	.265
2.....080	.100
3.....	.680	.580	.280
4.....	.812	.500	.435
9.....007	.020
10.....	.005	.004	.022
12.....014	.012
13.....	.072	.032	.077
14.....	.006	.002	.010
15.....	.168	.056	.072
16.....	.013	.040	.024
17.....	.433	.100	.283

These experiments seem to show a marked permeability of agar for iodine. In experiment No. 4, for example, although the tissues were examined only one hour after injection of the iodide, yet even this quickly the avascular agar contained as much iodine as the liver, which in this case gives an abnormally high figure because the animal could not be bled. In eight of 12 experiments, the agar contains a larger proportion of iodine than the liver, in only one is it considerably less. It will be noticed, also, that in this series as in all the other experiments the form in which the iodine is introduced seems to make little difference in its distribution.

As is to be expected, inflammatory exudates are prone to approach the blood in iodine content. Table 9 shows no evidence of any selective tendency of iodine to enter the inflammatory exudate, however, there nearly always being somewhat less iodine in the exudate than in the blood. The presence of iodine in the exudate would seem in all cases to be dependent entirely on simple diffusion, as in the case of necrotic tissues and implanted agar.

The high iodine content of tuberculous eyes is presumably to be explained, therefore, as due in part to the inflammatory exudate present in these eyes, and probably in less degree, to the necrosis in the tuberculous tissues.

Similarly, compression atelectasis of the lung, produced by pleural exudates, with the resulting greater or less edema and

inflammatory exudate in the alveoli, is associated with a corresponding slight increase of iodine in the injured lung, as shown in Table 10.

TABLE 9.
IODIN IN EXUDATES.

No. in Table 2	Blood	Liver	Exudate	Character of Exudate
1.....	.488	.101	.221	Edema about agar
4.....	.812	.500	.503	Subcutaneous abscess
6.....	.425	.125	.150	Serofibrinous, pleural
7.....	.391	.720?	.343	"
8.....	.305	.100	.310	Edema, subcutaneous
			.361	Pleural fluid
			.461	" fibrin
11.....	.019	.007	.020	Aleuronat subcutaneously
			.014	Edema, subcutaneous
13.....	.072	.032	.078	Serofibrinous pleuritis
14.....	.006	.002	.0035	"
			.0025	Edema, subcutaneous
15.....	.165	.059	.130	"
			.105	Aleuronat, intraperitoneal
16.....	.130	.040	.090	Pleural fluid
			.067	" fibrin
17.....	.433	.100	.336	Serofibrinous pleuritis

TABLE 10.
IODIN IN NORMAL AND COLLAPSED LUNGS.

No. in Table 2	Blood	Normal Lung	Collapsed Lung
6.....	.425	.147	.040?
8.....	.305	.189	.326
13.....	.072	.071	.088
16.....	.013	.020	.019
17.....	.433	.230	.320

These observations would seem to explain entirely, and on a simple physical basis, the observation of Bondi and Jacoby, Fillipi and Nesti, and Loeb, that drugs tend to enter inflammatory exudates. They enter simply because there is present an exudate which offers no resistance to their permeation to establish an osmotic equilibrium with the blood, and not because there is a specific affinity between pathological tissues and blood. Therefore, we are led to the conclusion that *the supposed affinity of certain drugs for certain pathological tissues merely depends on a decreased impermeability of the diseased cells, or diffusion into inflammatory exudates present in the diseased area, or both.* If this is the case, we might expect a non-diffusible colloidal substance to be unable to penetrate avascular diseased areas which are highly permeable for crystalloids, and this was found to be true. Tubercles, where there is no

blood supply, are relatively or absolutely impermeable, to foreign proteins present in the blood. This was shown in the following series of experiments.

ENTRANCE OF EGG ALBUMEN INTO TUBERCLES.

In order to determine the entrance of proteins into tubercles, advantage was taken of the delicate and accurate method offered by the anaphylaxis reaction for the detection of small quantities of foreign proteins. The following experiments were performed:

Experiment 1.—A large guinea-pig, which had been inoculated subcutaneously with 0.01 mg. of human tubercle bacilli three months previously, was bled to the amount of 4 c.c., and an equal amount of fresh filtered four per cent solution of Merck's dried egg albumen powder was injected into the blood by the intracardiac route. After three hours the animal was bled to death and the blood defibrinated. Autopsy showed large caseous cervical glands and smaller tuberculous mediastinal and inguinal glands. The spleen was studded with tubercles, many of which were two to four millimeters in diameter. The liver and lungs showed a few tubercles one to two millimeters in diameter. Samples were taken aseptically from various tissues, ground up with quartz sand, emulsionized under aseptic precautions in 10 c.c. of water for each gram of substance. After filtering, the extract was injected intraperitoneally in various sized doses into 22 guinea-pigs. Most of these pigs died in four to six days with a peculiar gelatinous exudative peritonitis, great numbers of cocci being present in this exudate. Eighteen days after injecting the tissue extract, the survivors received by the peritoneal route an injection of 0.05 gm. Merck's egg albumen, to discover whether the tissue extracts had contained sufficient egg albumen to sensitize the injected pigs. The results were as follows:

Sensitizing Dose			Result of Second Injection		
1.	5	c.c. blood (undiluted)	Died in 18 minutes		
2.	1	c.c. " "	"	"	15 "
3.	0.1	c.c. " "	"	"	20 "
4.	0.5	c.c. urine (undiluted)	"	"	45 "
5.	4	c.c. spleen extract	Severe symptoms, temperature fell to 100°		
6.	1	c.c. " "	"	"	" " 96°
7.	1	c.c. extract of caseous glands	Slight	"	" " from 104° to 101°
8.	0.75	c.c. " " tubercles from liver	No definite symptoms. No fall in temperature		
9.	0.50	c.c. " " " " "	No definite symptoms. Temperature 101°		

On account of the large proportion of deaths from infection, the results of this series were not altogether satisfactory, but such experiments as could be completed indicated that there was less

sensitization by extracts of tuberculous liver tissue and caseous glands than by extracts of spleen tissue taken from between the tubercles.

Experiment 2.—In the second experiment the danger of peritonitis was avoided by making the sensitizing injections subcutaneously. A 400 gm. guinea-pig which had large caseous inguinal glands from injection of human tubercle bacilli, was given an injection of three cubic centimeters of a four per cent solution of egg albumen powder in the carotid artery. This animal was very sick when injected, and it was so nearly dead three hours later when bled to death that it could be but partly bled, only five to six cubic centimeters escaping; therefore, all the organs were left containing much blood, including the caseous lymph glands, the necrotic content of which, when removed by scraping, was somewhat blood tinged. The tissues and blood were extracted with 10 parts of water as before, and the extract was injected in doses of from one to five cubic centimeters into 19 guinea-pigs. In this experiment, the sensitizing dose chosen was evidently too large, since 18 of the animals reacted fatally to egg albumen 18 days later. It can only be stated that the animals sensitized with extracts of the caseous material did not die as quickly as the others, and the sole survivor was in this group. Presumably, the amount of blood present in the tissues was sufficient to produce a fatal sensitization in the doses used.

Experiment 3.—The difficulties disclosed in the two previous experiments were avoided in a third trial. Here a 400 gm. pig with a large mass of fluctuating tuberculous lymph glands received two cubic centimeters of a four per cent solution of Merck's egg albumen in the jugular vein. Three hours later it was bled to death, but bled poorly and much blood was left in the body. The liver was found riddled with small tubercles, the spleen was greatly enlarged and contained some good-sized necrotic areas. The inguinal glands contained a great amount of soft caseous material, part of which was removed in two separate portions without pressure and without appreciable contamination with blood. Specimens of blood, liver tissue, spleen tissue between the large tubercles, the two separate lots of caseous material, and the uncaseated peripheral gland substance itself were each ground with quartz sand, extracted six hours with repeated stirring in 10 volumes of sterile water, filtered, and the filtrate injected subcutaneously into guinea-pigs. After 18 days, each pig was injected intraperitoneally with .050 gm. Merck's egg albumen, with the results shown in the table on p. 371.

From these experiments, it seems evident that the egg albumen present in the circulating blood does not enter the caseous material which is shut off from the blood by proliferating tissue, during three hours after its intravascular injection, at which time the blood contains sufficient egg albumen to sensitize a guinea-pig when injected in a dose of 0.001 c.c.¹ It is not possible to tell whether

¹ It may be mentioned that our experiments differ radically in one result from those of Vaughan, Cumming, and McGlumphy (*Ztschr. f. Immunitätsf.* 1911, 9, p. 16), for they state that egg-white injected into the blood of rabbits disappears in one hour, although it may be found in the various organs after that time. We found that egg albumen injected into the blood of guinea-pigs remains in the blood at least three hours, when 0.001 c.c. of blood contains a sensitizing dose. How much longer than three hours the albumen remains in the blood, and how much smaller doses than 0.001 c.c. are capable of sensitizing, we did not determine.

the positive results obtained with the liver, spleen, and tuberculous glands depend on the egg albumen contained within the cells or that present in the blood in these tissues. We were unable to secure a sufficient number of guinea-pigs to investigate this point.

1.	Blood		1 c.c.	Died in 20 minutes
2.	"		0.1 c.c.	Severe reaction
3.	"		0.02 c.c.	" "
4.	"		0.01 c.c.	" "
5.	Liver		2.0 c.c.	Moderately severe reaction
6.	"		0.5 c.c.	Died after 2 hours
7.	"		0.05 c.c.	" " " "
8.	Spleen		1 c.c.	Moderate reaction
9.	"		0.1 c.c.	Slight "
10.	"		0.01 c.c.	" "
11.	Gland tissue		1 c.c.	Died in 30 minutes
12.	" "		0.1 c.c.	Moderate reaction
13.	" "		0.01 c.c.	" "
14.	Caseous material	Sample A.	2.0 c.c.	No reaction
15.	" "	" "	0.5 c.c.	Slight or doubtful reaction
16.	" "	" "	0.1 c.c.	Doubtful reaction
17.	" "	" "	0.01 c.c.	No reaction
18.	" "	Sample B.	2.0 c.c.	Doubtful reaction
19.	" "	" "	0.5 c.c.	No reaction
20.	" "	" "	0.1 c.c.	" "
21.	" "	" "	0.01 c.c.	" "

SUMMARY.

A systematic consideration of the chemotherapy of tuberculosis rests on an investigation of the permeability of both the tubercle bacillus and the tuberculous lesion for chemical substances of different characters. It is shown that compounds of iodine injected into tuberculous animals enter glandular tubercles with readiness, so that the proportion of iodine in such tubercles is usually greater than it is in most other tissues except the kidney; furthermore it is greater in the caseous contents than in the cellular peripheries of the tubercles. Tuberculous eyes usually contain much more iodine than their normal mates. This property is shown not to depend on any specific character of the tubercle itself, for other

necrotic tissues also take up more iodine than normal tissues. The explanation offered is that normal cells are not perfectly permeable to iodides (except perhaps kidney cells) and lose this impermeability or semi-permeability when killed or injured, thus becoming entirely permeable for crystalloids present in the surrounding fluids. As the iodine content of the blood increases and decreases with absorption and elimination, so the iodine in the necrotic area, whether tuberculous or otherwise, varies, indicating an absence of any chemical or physical binding of the iodine in such areas. A simple, inert, colloid agar, implanted in the tissues, behaves in quite the same way.

Egg albumen injected into tuberculous pigs is found, by means of the anaphylaxis reaction, to penetrate the avascular tubercles but little if at all, even when present in the blood in large amounts. This agrees with the hypothesis that the passage of iodine from the blood into the tubercles is a purely physical matter, the crystalloidal iodine compounds diffusing through the inert colloidal solution of a necrotic area practically unimpeded, while the colloidal egg albumen, according to the law of colloidal diffusion, is practically unable to diffuse through such a colloidal solution.

No evidence could be found of any tendency for iodine compounds of whatever nature to accumulate in tubercles or other necrotic areas, or to persist in such areas when disappearing from the normal tissues and the blood.

Exudates contain approximately the same proportion of iodine as the blood of the same animals, and hence any area with inflammatory edema and congestion will commonly show more iodine than normal tissues, although not usually more than the blood. No evidence was found of any specific entrance or fixation of iodine in inflammatory exudates. The iodine is distributed about alike in the fluid and solid portions of the exudate, indicating simple diffusion. Of normal tissues only the kidney seems to contain approximately as much iodine as the blood of the same animal. This may have some bearing upon its excretory function, since it indicates a greater permeability of renal cells than of other gland cells for iodides.